Inhibition of Tryptophan Pyrrolase Induction by Carbon Tetrachloride in Rats

RAYMOND D. MAGUS[1] AND JAMES R. FOUTS

Department of Pharmacology, University of Iowa College of Medicine, Iowa City, Iowa 52240

(Received February 26, 1968, and in revised form June 3, 1968)

SUMMARY

The inducing action of hydrocortisone and L-tryptophan on hepatic tryptophan pyrrolase in rats treated previously with CCl_4 was studied. CCl_4 exhibited a dose-dependent (and time-dependent) inhibitory action on the hematin-activated (apoenzyme) form of tryptophan pyrrolase but had no effect on basal holoenzyme activity. A low dose of CCl_4 (0.1 ml/kg) abolished induction of the holoenzyme by hydrocortisone but had little effect on the tryptophan-induced holoenzyme or total enzyme activity. This inhibitory action of CCl_4 required between 6 and 12 hr of exposure of the animal to CCl_4 *in vivo*, i.e., prior to administration of the inducing agents. A 10-fold increase in CCl_4 dose to 1.0 ml/kg did not abolish the inducing action of tryptophan; however, the enzyme response to tryptophan administration was appreciably reduced in rats receiving the higher CCl_4 dose.

INTRODUCTION

Tryptophan pyrrolase (EC 1.13.1.12) activity in rat liver is regulated by separate mechanisms in response to administration of hydrocortisone or L-tryptophan (1). Induction of this and other enzymes in the liver of the rat by hydrocortisone appears to be dependent on coordinate synthesis of RNA (2–4) and is accompanied by concomitant increases in immunologically reactive enzyme protein (5, 6). The enhanced activity of hepatic tryptophan pyrrolase in the rat following administration of L-tryptophan is also accompanied by an increase in enzyme protein (5, 6), although the increase in enzymic activity detectable *in vitro* arises from a combination of several factors. The enhanced tryptophan pyrrolase activity seen following substrate administration *in vivo* is attributed to a decrease in the rate of enzyme degradation (6, 7), activation of apoenzyme ("cofactor induction") (8) resulting from induction of hepatic aminolevulinate synthetase activity (9), and "induction" by a process sensitive to inhibitors of messenger RNA translation (2, 10) and to purine and pyrimidine antimetabolites (11, 12). The basic difference between hydrocortisone and tryptophan "induction" of hepatic apotryptophan pyrrolase is that hydrocortisone produces new sites for enzyme synthesis, whereas tryptophan utilizes pre-existing templates (1).

The apparent difference in site and mechanism of action of hydrocortisone and tryptophan was illustrated further in a previous study from this laboratory. Rats treated for 12 hr with SKF 525-A (2′-diethylaminoethyl-2,2-diphenylpentanoate-HCl) showed a marked reduction in ability of their livers to synthesize tryptophan pyrrolase in response to a challenging dose of hydrocortisone. On the other hand, the hepatic response to administration of tryptophan was little affected by treatment with SKF 525-A (13).

The interaction of SKF 525-A with hepatic ergastoplasm bears some resemblance to that of CCl_4. Both agents affect the membranous ultrastructure of the hepatic parenchymal cell (14–16) and

[1] Present address: Center for Research in Pharmacology and Toxicology, University of North Carolina, Chapel Hill, North Carolina 27514.

inhibit hepatic microsomal drug-metabolizing enzymes (17, 18). Both SKF 525-A and CCl_4 produce fatty infiltration of the liver (19, 20), hepatic glycogen depletion (16, 21, 22), and inhibition of protein synthesis (23, 24). The present experiments describe another similarity in the effects of CCl_4 and SKF 525-A on the liver, namely, a selective inhibition by CCl_4 treatment of the induction of hepatic tryptophan pyrrolase. Carbon tetrachloride blocked the induction of the enzyme by hydrocortisone but not by tryptophan administration.

METHODS

Male Sprague-Dawley rats (weight range, 290–310 g) were used in all experiments. These rats were obtained from Simonsen Laboratories, Minneapolis, and were allowed free access to food and tap water at all times. Control rats were killed at the same times as treated animals. After the animals had been killed by a blow on the head, livers were quickly excised and frozen on Dry Ice.

All subsequent preparative procedures were performed at ice-bath temperatures. Homogenization of livers was performed in a Teflon-glass (Potter-Elvehjem type) homogenizer with two excursions of the pestle. For routine assay of hepatic tryptophan pyrrolase, each gram of liver was homogenized in 3 ml of ice-cold 0.15 M KCl containing 2.5 mM NaOH and 1.0 mM disodium EDTA (25, 26). This homogenate was centrifuged in the cold for 20 min at 9000 *g* (or, where noted, at 105,000 *g* for 60 min). One-half milliliter of 9000 *g* supernatant fraction, equivalent to 125 mg of liver, was incubated (under O_2 and with shaking at 37°) in triplicate, with each beaker containing the following components: phosphate buffer, 0.125 M, pH 7.0; ascorbic acid, 10 mM; and L-tryptophan, 3 mM (25, 27), in a final volume of 4.0 ml. Hematin was prepared immediately before use as described by Knox and Ogata (27). For routine assays a final hematin concentration of 10 μM was used (Fig. 1). Following 20 min of incubation, one beaker (blank) was deproteinized with 15% metaphosphoric acid (25). It was found that blanks incubated in the absence of added substrate showed a negligible increase in optical density at 365 mμ following this time period. The remaining beakers were incubated for an additional 60 min, during which enzyme activity was linear with time, and were then similarly deproteinized. The optical density (365 mμ) difference between the neutralized acid-soluble fractions from the 20- and 80-min incubations was taken as an index of the enzymic formation of kynurenine. One unit of enzyme catalyzed the formation of 1 μmole of kynurenine (25).

All drugs were administered by intraperitoneal injection. SKF 525-A (Smith Kline and French Laboratories) and hydrocortisone 21-phosphate disodium (Hydrocortone, Merck and Company) were administered in aqueous solution. L-Tryptophan (Nutritional Biochemicals Corporation) was initially dissolved in a minimal amount of 0.01 N NaOH and was injected immediately following dilution with an appropriate volume of water. Carbon tetrachloride (reagent grade) was administered in a corn oil (USP) solution. Student's *t*-test was used to test for significant differences between means.

RESULTS

Effect of hematin on tryptophan pyrrolase activity in vitro. A study was performed in order to determine the optimal concentration of hematin required for maximal activation of tryptophan pyrrolase *in vitro*. Enzyme assays were conducted with 9000 *g* or 105,000 *g* supernatant fractions from liver homogenates prepared from rats treated with hydrocortisone (30 mg/kg i.p. for 6 hr). It was found that the concentration of hematin required for maximal activation of tryptophan pyrrolase in the 9000 *g* supernatant fraction was 10 μM, whereas peak enzymic activity in the soluble fraction (105,000 *g* supernatant) was obtained at a hematin concentration of approximately 1 μM (Fig. 1). Since all subsequent tryptophan pyrrolase assays were performed on 9000 *g* supernatant fractions from liver homogenates, a 10 μM concentration of hematin

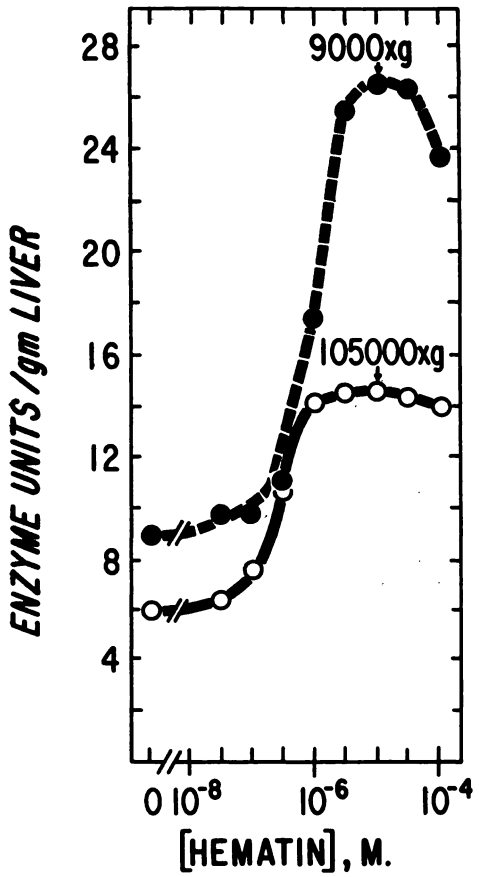

FIG. 1. *Effect of hematin addition in vitro on tryptophan pyrrolase activity in subfractions of liver homogenates obtained from rats treated with hydrocortisone*

The concentration of hematin is expressed on a $\log_{10}$ scale. Livers were pooled from rats weighing 300 ± 10 g given 10 mg of hydrocortisone intraperitoneally 6 hr before assay.

was selected as optimal for the detection of apparently unconjugated apoenzyme (28). This concentration of hematin was also optimal for 9000 *g* preparations obtained from CCl_4-treated rats.

Effects of CCl_4 on basal apo- and holotryptophan pyrrolase activity in rat liver 9000 g supernatant fractions. The appearance of biochemical lesions characteristic of CCl_4-induced hepatic dysfunction is dependent, among other factors, on the time of treatment and on the dose of CCl_4 given (29, 30). Since tryptophan pyrrolase of rat liver turns over quite rapidly, with an estimated half-life of the order of 2 hr (6, 12, 31, 32), it might be expected that CCl_4, an inhibitor of protein synthesis (20), would affect basal enzyme levels.

It can be seen in Table 1 that basal holo tryptophan pyrrolase activity (i.e., tryptophan pyrrolase assayed in 9000 *g* supernatant fractions not supplemented with hematin) was not affected by treatment *16* hr previously with either 0.1 or

TABLE 1

Effects of different doses of CCl_4 on total and holoenzyme tryptophan pyrrolase activity 16 hr after treatment with CCl_4

CCl_4 was dissolved in corn oil. The volume of CCl_4–corn oil solution injected intraperitoneally was kept at 5 ml/kg of body weight. Rats weighed 300 ± 10 g. Corn oil alone had no effect on enzyme activity measured with or without added hematin. Hematin, when used, was added at a final concentration of 10^{-5} M. Values are mean ± standard error with $N = 4$.

Dose of CCl_4 i.p.	Tryptophan pyrrolase activity	
	Without hematin (holoenzyme)	With hematin (total enzyme)
ml/kg	*units/g liver*	
0	2.8 ± 0.4	6.8 ± 0.4
0.1	3.0 ± 0.2	5.3 ± 0.1[a]
1.0	2.4 ± 0.3	3.7 ± 0.3[a]

[a] Significantly different from corn oil-treated group assayed with hematin ($p < 0.05$).

1.0 ml (1.0 or 10 mmoles) of CCl_4 per kilogram administered intraperitoneally. However, total tryptophan pyrrolase activity (i.e., tryptophan pyrrolase assayed in the presence of added 10 μM hematin) was inhibited in a dose-dependent manner by CCl_4 treatment (Table 1). This inhibitory action of CCl_4 on the hematin-activated enzyme activity was not seen following treatment 10 hr previously with a dose of 0.1 ml of CCl_4 per kilogram (Table 2, line 5 compared with line 3).

Effects of CCl_4 on hepatic responsiveness to hydrocortisone or L-tryptophan as inducers of tryptophan pyrrolase. The response of hepatic tryptophan pyrrolase to treatment 4 hr previously with hydrocortisone was not altered in corn oil-treated

Mol. Pharmacol. 4, 465–470 (1968)

TABLE 2

Effects of CCl_4 on the induction of hepatic tryptophan pyrrolase by hydrocortisone or L-tryptophan

CCl_4 was dissolved in corn oil. All injections were made intraperitoneally. Rats weighed 300 g. Corn oil or CCl_4 was given 10 or 16 hr before, and enzyme inducers were given 4 hr before, the animals were killed for enzyme assay. Hydrocortisone (10 mg/rat) and L-tryptophan (150 mg/rat) were injected as aqueous solutions. Total enzyme was assayed in the presence of 10^{-5} M hematin. Enzyme activity is mean ± standard error with $N = 4$. Values in parentheses represent percentages of control values. A plus sign means treatment with the agent in the column heading, while a minus sign means no treatment or treatment with water. Lines 1–6 contain data from a single experiment, while lines 7–18 are data from another experiment.

	Agent, dose, and time until death					Enzyme inducer given 4 hr before death		Hepatic tryptophan pyrrolase activity			
	Corn oil		CCl_4								
			0.1 ml/kg					Without hematin (holoenzyme)		With hematin (total enzyme)	
Line	1 ml/kg 10 hr	5 ml/kg 16 hr	10 hr	16 hr	1 ml/kg 16 hr	Hydrocortisone	L-Tryptophan	*units/g liver*	%	*units/g liver*	%
1	–	–	–	–	–	–	–	3.1 ± 0.3	(100)	8.0 ± 0.6	(100)
2	–	–	–	–	–	+	–	6.2 ± 0.8	(200)[a]	22 ± 0.4	(280)[a]
3	+	–	–	–	–	–	–	3.2 ± 0.2	(100)	9.7 ± 0.4	(100)
4	+	–	–	–	–	+	–	5.8 ± 0.3	(180)[a]	19 ± 0.8	(195)[a]
5	+	–	+	–	–	–	–	3.1 ± 0.3	(100)	9.1 ± 0.4	(100)
6	+	–	+	–	–	+	–	5.6 ± 0.6	(180)[a]	20 ± 2.0	(220)[a]
7	–	–	–	–	–	–	–	2.5 ± 0.2	(100)	5.6 ± 0.4	(100)
8	–	–	–	–	–	+	–	5.9 ± 0.8	(235)[a]	15 ± 1.3	(230)[a]
9	–	–	–	–	–	–	+	8.5 ± 0.5	(340)[a]	20 ± 0.9	(360)[a]
10	–	+	–	–	–	–	–	2.8 ± 0.4	(100)	6.8 ± 0.4	(100)
11	–	+	–	–	–	+	–	6.6 ± 0.8	(240)[a]	16 ± 2.8	(235)[a]
12	–	+	–	–	–	–	+	10.2 ± 1.3	(360)[a]	21 ± 1.8	(310)[a]
13	–	+	–	+	–	–	–	3.0 ± 0.2	(100)	5.3 ± 0.1	(100)
14	–	+	–	+	–	+	–	3.7 ± 0.3	(120)	12 ± 1.2	(230)[a]
15	–	+	–	+	–	–	+	11.9 ± 1.4	(400)[a]	21 ± 2.2	(400)[a]
16	–	+	–	–	+	–	–	2.4 ± 0.3	(100)	3.7 ± 0.3	(100)
17	–	+	–	–	+	+	–	2.2 ± 0.3	(90)	5.0 ± 0.8	(135)
18	–	+	–	–	+	–	+	4.9 ± 0.2	(205)[a]	7.4 ± 0.6	(200)[a]

[a] Significantly different ($p < 0.05$) from control value given in the same group in the same column.

controls or in animals which had received an injection of CCl_4 (0.1 ml/kg) 6 hr prior to the hydrocortisone (Table 2, lines 1–6). If the time between CCl_4 administration and hydrocortisone treatment was extended to 12 hr, the "inducing" action of hydrocortisone on holotryptophan pyrrolase was abolished by the lower dose (0.1 ml/kg) of CCl_4 (Table 2, lines 13 and 14 vs. 10 and 11). This dose regimen had little *inhibitory* effect on *total* enzyme induction by hydrocortisone, nor did CCl_4 block the effect of tryptophan on either form of the enzyme (Table 2, line 15 vs. line 12). A 10-fold increase in CCl_4 dosage (to 1.0 ml/kg, 16 hr) abolished the "inducing" action of hydrocortisone on either form of the enzyme (Table 2, lines 16 and 17 vs. 10 and 11) and reduced the effectiveness of tryptophan in enhancing enzyme activity by approximately 50% (Table 2, lines 16 and 18 vs. 10 and 12). In no case did corn oil treatment alone affect enzyme activity (Table 2, lines 10–12). Thus, in spite of the hepatic necrotizing action of this massive dose of CCl_4 (1.0 ml/kg), the animals retained some ability to increase their hepatic tryptophan pyrrolase activity in response to administration of L-tryptophan.

DISCUSSION

The structural damage to the hepatocyte produced *in vivo* by cleavage products of CCl_4 affects both membranous and ribosomal components of the hepatocyte ergastoplasm (24, 33, 34). CCl_4, in doses much greater than those used in the present study, produces polyribosome destruction, with a shift in the hepatic polysome profile toward a larger number of monosomes (35, 36). Furthermore, it has been suggested that CCl_4 may have a destructive action on endogenous hepatic messenger-like RNA (37, 38).

Our experiments indicate that CCl_4 treatment (at least in the lower dose used) need not produce detectable destruction of endogenous (pre-existing) enzyme-synthesizing sites (i.e., polysomes). This conclusion comes from our finding that tryptophan retained its ability to increase hepatic tryptophan pyrrolase activity in animals which had received CCl_4 (0.1 ml/kg) 12 hr before tryptophan (Table 2, line 15 vs. line 12). With the higher CCl_4 dose (1.0 ml/kg), however, some inhibition of the response to tryptophan was seen (Table 2, line 18 vs. line 12). This dose-dependent effect of CCl_4 on the induction of tryptophan pyrrolase by tryptophan could be the result of a dose-dependent effect of CCl_4 on polysome breakdown; i.e., perhaps only large doses of CCl_4 produce marked polysome destruction.

The effects of CCl_4 on tryptophan pyrrolase induction by tryptophan as compared with hydrocortisone may be due to different mechanisms. Treatment with CCl_4 may block the inducing action of hydrocortisone by interfering with the synthesis *de novo* or with the utilization (e.g., membrane association) of new polysomes formed in response to hydrocortisone treatment (39). The latter effect could result from the degradative action of CCl_4 on the lipoprotein matrix of the ergastoplasm (20, 33), thereby preventing interaction of new polysomes with these membranous components (40, 41). If this were the mechanism whereby CCl_4 prevented the inducing action of hydrocortisone, then many RNA-dependent hepatic enzyme inductions might be affected by CCl_4. The experiments of Fujimoto and Plaa (42) lend support to this possibility; i.e., CCl_4 may be a general inhibitor of RNA-dependent enzyme inductions. These investigators found that the phenobarbital-induced decrease in hexobarbital sleeping times in rats was prevented by prior treatment with CCl_4 or by simultaneous doses of CCl_4 (approximately 0.1 ml/kg). The effect of phenobarbital on hexobarbital sleeping times in rats has been attributed to induction of hepatic microsomal hexobarbital-metabolizing enzymes via genomic activation (43).

Our data also support the possibility that CCl_4 treatment enhanced the rate of breakdown *in vivo* of apoenzyme but not holoenzyme. Under such a condition, the rate of apoenzyme breakdown could exceed that of apoenzyme synthesis and give the results reported in Table 1. The fact that

tryptophan could cause increased holo- and total enzyme activity in animals treated with CCl_4 (Table 2, lines 15 and 18) may have resulted from this substrate protecting (*in vivo*) against the destructive action of CCl_4 on unconjugated apoenzyme. High doses of CCl_4 may partially overcome this substrate protection (Table 2, line 18 vs. line 15).

Other factors, such as a difference in accessibility of hydrocortisone and tryptophan to their sites of action in normal as compared with CCl_4-treated animals, cannot be ruled out by these experiments.

ACKNOWLEDGMENT

This investigation was supported by Grants GM12675 and 5-T01-GM 01308 from the National Institute of General Medical Sciences.

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